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Nano Micelle: Novel Approach For Targeted Ocular Drug Delivery System

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Abstract
Ocular delivery like all other systems is challenging for the researchers as one can instill drug into the eye but can never ensure the correct amount reaches the eye. Ophthalmology for all these reasons has commenced its dependence on novel techniques. Ocular through novel becomes a boon as you can deliver huge amounts or you can deliver as per the need but it's always a tricky one as you need more residence of the drug in the eye for better action, but the lacrimal drainage and rejuvenation spoils all novel thoughts and leads the novel with surface modification. The surface of the particles needs to be adhering to the surface of the eye or in the cul de sac as with more drug amount we need more residence for action.
This research work provides a better way for ocular drug delivery to the eye through a novel system, i.e., Nano micelle which consists of tri-block copolymerization with fluoroquinolones for treating gall bacterial infections of the eye. The work is based on how we have employed the nano micelle of a fluoroquinolone for ocular delivery that adheres in the eye and delivers the drug for a period more which cannot be expected through the conventional system.

Keywords: Ocular Drug Delivery, Nano micelle, Polymers.

1. Introduction

The eye is a perplexing organ with special anatomy and physiology. The structure of the eye can be divided into two major sections or parts: The anterior portion and the posterior section. The anterior section of the eye involves nearly 33% while the rest of the segment is involved by the posterior portion. Tissues, for example, conjunctiva, cornea, watery diversion, ciliary body, iris and focal point make up the anterior segment. The posterior section of the eye includes the sclera, retinal shade epithelium, choroid, neural retina, vitreous humour, and optic nerve. The posterior and anterior fragment of the eye is influenced by different vision compromising sicknesses. Diseases influencing the anterior portion include, yet are not restricted to glaucoma, unfavourably susceptible conjunctivitis, cataract and anterior uveitis. Although diabetic retinopathy and age-related macular degeneration (AMD) are the most common diseases affecting the posterior region of the eye. Any vision-threatening ocular infections, for example, age-related macular degeneration (AMD), glaucoma, diabetic retinopathy and proliferative vitreoretinopathy may influence visual impairment. Ocular medication conveyance explicitly to the intraocular tissues remains a provocative undertaking because of the occurrence of different physiological hindrances [1]. The novel technology was developed for all other body systems but has now gained more paces for ocular drug delivery, starting from microparticles to liposomes to nanoparticles. They deliver the desired amount with less loss and provide more prolonged the action.

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Nano micelle comprises amphiphilic particles that self-amass in fluid media to get in the shape of an organized supramolecular system. Micelles can be set up in different sizes (10-100nm) and shape contingent on the sub-atomic weights of the corona and core framing blocks. Nano micelle has been an alluring transporter for its capability to solubilize hydrophobic particles in an aqueous arrangement [3, 4]. The cohesive nature of the polymeric micelles concluded in upgraded contact with the visual surface while their little size permits more tissue penetration. In particular, being very soluble in water, these polymeric micelles produce a clear aqueous solution that permits simple application as eye drops with no vision obstruction. Improved strength, bigger cargo limit, non-toxic, easy surface adjustment & controlled medication discharge are extra favourable circumstances with polymeric micelles [5].

It is regular learning that the ocular bioavailability of medicaments topically applied as eye drops is low. The ingestion of medicaments in the eye is seriously constrained by some defensive mechanisms that guarantee the best possible functioning of eyes and by other factors such as:Instilled solution elimination, Tear turnover and lacrimation, Digestion, Evaporation of tear, non-beneficial adsorption/absorption and Restricted corneal zone &poor corneal.

The elimination of the dose administered by means of the nasolacrimal framework into the nasopharynx and GIT happens when the volume of humour in the eye surpasses the ordinary lacrimal volume of 7 to10 microliters. Along these lines, the part of the instilled portion (one to two drops, relating to 50-100 microliters) that isn't dispensed with by spillage from the palpebral crevice is drained rapidly & time of contact of dose with absorbing surfaces (sclera and cornea) is decreased to a limit of two minutes [6].

The lacrimation and physiological tear turnover (16%/min in people in typical conditions) can be expanded and stimulated by the instillation even of somewhat disturbing solutions. The net outcome is a dilution of the medication applied drug loss acceleration.

It is conclusively settled that the rate at which instilled solutions are expelled from the eye, fluctuates directly with the volume of instilled. Or we can say that the bigger the instilled volume, the more quickly the instilled solution is eliminated from the pre-corneal zone [7].

Nano micelle comprises amphiphilic atoms that for the most part self-amass in fluid media to frame organized supramolecular structures. Micelles are framed in different sizes (10-100nm) and shapes (circular, cylindrical, star-moulded, and so on) depending upon the sub-atomic weights of the corona & core framing blocks. The self-gathering occurs above at a certain concentration, alluded to as CMC [8].

For common micellar structures the force driving self-assembly and support of supramolecular assembly is hydrophobic associations of centre framing hinders. The crown shaping square is soluble in water that inhibits micelles dissolvable in the watery stage. Exploiting hydrophobic centres nanocarriers may be used to upgrade the water solubility of hydrophobic atoms.

Nano micelles explored for ODD up to this point be classified into 3 general classes, i.e.

1. Polymeric Nano micelle (Used for ophthalmology)
2. Surfactant Nano micelle
3. Poly-ionic Nano micelle [9]

Drug:

Name of the class: Fluoroquinolone

Chemical Name of the class: 6-fluoro-8-methoxy-4-oxoqu—inoline- 3-carboxylic acidMolecular formula: $\mathbf{C_{10}H_2FN_3O_3.R_4}$Molecular weight: 401.431 g/mol Structure: Class structure; Fig 1 Physical description: It is a mono-hydrochloride which is a slightly yellow to yellow crystalline substance. Category: Antibiotic Solubility: Soluble in water, highly soluble in ethanol, sparingly soluble in acetone, soluble in methanol and chloroform. Melting point: 242-245 °C [10, 11, 25, 41] Different other formulations can also be done by the above drug [42, 43, 44]. Dose and consumption usage [41, 27]: Drug is a fluoroquinolone derivative. It is a broad-spectrum antibiotic that works against all the gram -ve, gram +ve, and even anaerobic bacteria.

Its dose is 400 mg tablet oral or 400 mg/ 250 ml injection parenteral, once in day for 5 to 21 days depending upon the infection. In ocular its dose is one drop from a 0.1% solution in 10ml in infected eye 3 times a day which will dispense 10-50 mcl in the eye and continue it for 7 days. Antimicrobial action: Its antibacterial spectrum includes Gram (−ve) rods (Escherichia coli, Proteus species, klebsiella

species), Haemophilus Influenzas a typical bacterium (Mycoplasma, Legionella, Chlamydia), and Streptococcus pneumoniae, and anaerobic bacteria. [20, 22]

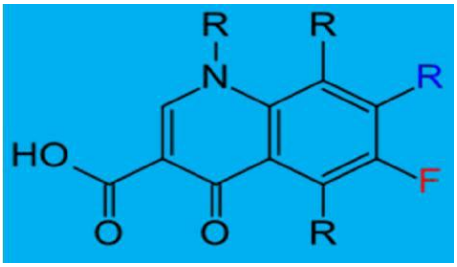

Fig 1: Structure of Fluoroquinolone moiety

Pharmacokinetic property:

Absorption: Readily absorbed from the GIT with a bioavailability of 90% with standard deviation of 1.45-1.48. While in topical only 1-5% of the administered is absorbed from the layers of the eye.

Time for C max: Peak plasma concentration is reached within 1 to 3 hours after oral administration and 1hour after topical administration. C max: 4.5 ± 0.53 µg/mL (oral) and 0.1 + 0.05 µg/mL (topical) AUC: 48 ± 2.7 µghr/mL and 1.5 + 0.27 µghr/mL (topical) Volume of Distribution: 1.7-2.7L / kg (oral) Total Body and Renal Clearance: 12 ± 2 and 2.6 ± 0.5 L/hr (oral) Protein binding: 30 to 50% (oral) and 0.44 to 0.55% (topical)

Half-life: 11.5-15.6 hrs (oral) and 2-3 hrs (topical) (depend upon the dose administered)

Metabolism and Excretion [13]: Drug is metabolised to an N-sulphate conjugate and an acyl glucuronide inhuman. The N-sulphate and the unchanged drug are detected in plasma, urine and faeces. The acyl-glucuronide is detected in plasma and urine, but not in faeces. The urinary excretion of the unchanged drug accounts for 19-22% of the given dose. Neither renal nor hepatic impairment significantly affect the pharmacokinetics of drug [24].

Mode of action: This drug inhibits the topoisomerase II ligase domain while leaving the nuclease domains intact. This alteration, in combination with the continual action of topoisomerase II in the bacterial cell, results in DNA fragmentation via the nucleic activity of the intact enzyme domains. [12, 24] Adverse reactions: Irreversible peripheral neuropathy, spontaneous tendon rupture and tendonitis, hepatitis, psychiatric effects (hallucinations, depression), torsade de pointes, Steven-Johnson syndrome and clostridium difficile-associated disease (CDAD), and photosensitivity / phototoxicity reactions are all possible side effects of drug therapy. [24]

2. Experimental:

The samples of the research work were formulated through the finest equipment at the Formulation and Development Dept. of Venus Remedies and the developed samples were analysed by the Analytical Research Development Laboratory (ARD Lab) of Venus Remedies Ltd., SAIF at Punjab University, Instrumentation Lab, SRMS College of Pharmacy, Material Research Centre at MNIT Jaipur and Universal Labs, Karnataka.

Method of formulation:

1. Quality By design
2. Optimization of Formulation by using 2^3 Factorial Design

The various combinations created are there in Table1. [31, 33]

Nano micelle formulation:

Method: Solvent emulsification by using the tri block copolymer

The whole method is divided into two stages:

First Stage: Preparation of reagent solution

Preparation of Poloxamer Solution:

Poloxamer solution in two concentrations was created for the desired 8 formulations according to the 2^3 factorial design. The solution was prepared by weighing 1.4 gm of Polox 407, then adding 0.5 gm, followed by a gap, then 0.5 gm, followed by a gap, then 0.5 gm, and finally 0.4 gm after a gap to 5 ml water under magnetic stirring then adding the remaining 5 ml to the beaker after a period of 10 min and keeping it at 3-4 °C for 24 hours to get a clear solution. A similar technique was followed for the 28% solution, in which 2.8 gm of Polox 407 was accurately weighed and transferred to a beaker in three proportions: 1 gm then, with a gap 1gm, and finally 0.8 gm containing 5 ml water under magnetic stirring. The remaining volume of 5 ml was added after 10 minutes of stirring, and the beaker was held at 3-4 °C for 24 hours to obtain a clear solution. [38, 39]

Preparation of PVA Solution:

PVA solution was also prepared according to the opted 2^3 Factorial Design, where the 0.25% and 0.5% solution of PVA was prepared by addition of 0.25 gm. and 0.5 gm. to WFI, volume made up to 100 ml with WFI only, and left for stirring at 70-80 °C, till a clear solution was obtained. The formed solution was cooled to room temperature before being employed in the formulation.

Preparation of Chitosan Solution:

Chitosan solution was prepared in an acetic acid solution 3% v/v. Acetic acid 3% solution was made by transferring 3 ml Acetic Acid to a 100 ml volumetric flask and then raising the volume to 100 ml with WFI. Then chitosan solution was prepared by weighing 0.5 gm. of chitosan and transferring it into 3% AA solution on magnetic stirring for 3 to 4 hours

and then leaving it on overnight standing, to get a chitosan solution that has a little viscosity to that is why it is referred as chitosan solution.

Second Stage: Formulation stage

This stage is further divided into five stages:

First Stage: The Poloxamer, PVA, and Drug according to the 8 decided formulations from the 2^3 factorial designs were weighed and withdrawn [38, 41].

Second Stage: Then after that Poloxamer 28 or 14% solution was added with 0.2 mcl of Span 20, and kept on stirring. Simultaneously the selected drug amount was taken and was transferred into 5ml ethanol and a clear solution was made through stirring. Then this prepared solution was transferred to the above-prepared poloxamer solution on stirring, and allowed to stir for 2 hours. Froth is obtained it is set aside for 3 hrs. to get a cleat solution without froth.

Third Stage: Now the second solution of PVA according to the design selected was taken up to 10 ml and 0.2 mcl of formalin was transferred in it on stirring, following the addition of chitosan solution 10ml, and was allowed to form a clear solution for a span of 1 hr.

Fourth Stage: The above-prepared solutions were taken, and then poloxamer solution was added dropwise through a syringe to the PVA and Chitosan solution under magnetic stirring at a speed of 3000-4000 RPM, for a span of 10-12 hrs. This solution formed was then subjected to settle and give a clear solution without froth.

Fifth Stage: In this stage, the above-prepared solution was subjected to vacuum drying through the Heater and Dryer at the premise. The temp was set up to 38+ 2°C and a pressure of 0.2mbar. The product was left for overnight drying and then crushed to get a fine powder. The powder then obtained was subjected to evaluation to determine the various factors like particle size, entrapment etc.

Evaluation:

Preformulation studies:

a) Solubility:

The solubility study of the drug was performed by taking approximately 10 mg of drug and an equal volume of different solvents in the flask, following which the flask was shaken in a flask shaker for 24 hrs and the solubility was determined in comparison with each other, i.e. The clearest solution was said to be highly soluble and others in comparison to it were demarcated. [14, 15]

b) Melting Point:

The melting point was determined by using the Vigo melting point apparatus. The heating of oil and the heat generated in the apparatus is sufficient to measure the melting point in the range set by you which reaching maximum till 300 °C. The accuracy is between 0.3-0.5 °C and the heating rate of the equipment is 1 °C/min. [14, 15]

c) FTIR:

The drug for its qualitative analysis underwent FTIR examination. Here the drug approximately 1 mg was taken and was mixed 50-60mg of KBr. A proper physical mixture was made through titration but with a mild force as excessive force may cause the drug to disrupt up [14, 15].

d) UV- Spectrophotometric Analysis of drug:

In UV spectrophotometric analysis of the drug, the main aim is to check and verify that the product that we are using is free of impurities and is equal to the standard formulation values. The Shimadzu UV Spectrophotometer was used to record the absorbance and plot the calibration plot. [14, 15]

e) Partition Coefficient Method: The partition coefficient was determined by the shake flask method. Before this method, there is a need for one thing i.e., calibration plot of the drug in water. **[15, 16]**

Shake Flask Method:

In this method, approximately 10 mg of drug was taken and was poured in a flask containing 50 ml each of octanol and water. The flask was subjected to shaking in a shaker for a period of 24hrs until the equilibrium was reached. The flask is a separating funnel having a knob in the bottom was allowed to stand so that both the layers obtained are distinctly visible, then the lower layer being water was slowly withdrawn in another beaker and the upper layer in another beaker. After that, the lower layer was subjected to UV analysis, and the absorbance was noted.

Formulation evaluation:

The dried micelles obtained were subjected to the following mentioned evaluations:

a) Drug Entrapment

The drug entrapment refers to the amount of drug that has gone in the polymer matrix, and is freely dispersed in the core of the nano micelle. The reading for drug entrapment was taken by the UV apparatus and through the equation the value was noted. [21, 22]

$$\mathbf{DE} = \frac{\mathbf{Wt.\ of\ drug\ in\ nanomicelle\ calculated}}{\mathbf{Wt.\ of\ drug\ taken\ to\ formulate\ it}} * \mathbf{100}$$

Here,

DE: Drug Entrapment

Wt.: Weight

b) Drug Loading Capacity

The drug loading capacity is defined as the amount that is loaded in nano micelle and is in accordance with the amount that is used to formulate it. The drug loading is calculated by dissolving an accurate amount say 10 mg of nano micelle in STF and then allowing it to magnetically stir for 24 hrs and then recording the absorbance. [21, 22]

$$\text{DLC} = \frac{\textbf{Wt. of drug in nanomicelle calculated}}{\textbf{Wt. of nanomicelle taken for study}}$$

Here,
DLC: Drug Loading Capacity
Wt.: Weight

c) *In- Vitro* Drug Release:

The *in-vitro*release study of nano micelles of the drug was done in triplicate in STF having pH 7.4 + 0.5. The simulated tear fluid was freshly prepared by using sodium hydrogen carbonate, sodium chloride and calcium chloride as mentioned in the table. The pH was found to be 7.39. A dialysis bag of 10 cm was cut from the pile and was moistened in WFI. Nano micelles weighing equal to 10 mg were placed in each bag along with 1ml STF and were threaded at both ends to seal the bag. This bag was then poured in the dissolution flask containing 10 ml of STF equal to the final replenishment in the eye left after the cycle or equal to the total lacrimal fluid present at an instance in the eye. The study was done for four different types of nano micelles whose entrapment efficiency and loading capacity were higher than the rest. The study was carried for 24 hr. and the absorbance starting from 30 min to 60 then to 120 then to 180 and so on till1440 min was noted and the drug release was calculated. [21, 34]

d) Particle Size and Poly-dispersity:

Micelles obtained from the vacuum heating and drying were transferred into WFI and were sonicated for a period of 10 to 20 min to form a final dispersion. Then this dispersion was filtered through the membrane filter of 0.45mm pore size. The filtered solution was then sent for DLS through the Zeta Sizer at a temp of 25°C and at an angle of 90°, which was used to determine the average particle size. The particle size, zeta potential, and the polydispersity index that will be obtained through this evaluation will be a mean of 3 observations that will be taken through the DLS apparatus and will enable us to proceed further. [35, 36, 37]

e) Turbidity analysis:

The nano micelles prepared were analysed for turbidity, as turbidity is a major factor that needs to be analysed if we are employing the formulation of Ocular Drug Delivery. [22, 23]

Turbidity = (100 -%Transmittance)/100

All the readings were taken in triplicate, at three instances of 0 hr, 6hr, and 12 hr.

f) Morphology study of the Nano micelle formulation:

The he prepared nano micelle formulation was subjected to the TEM study. Here the dried micelles were transferred into STF and a nano-dispersion was made, it was then sonicated for 10 min. After sonication, a drop of it was taken and was applied on the disc of the gauge, which was immersed through a rod in the TEM apparatus, and the mesh was from the 400 meshes were selected, where the density of the nano micelle was maximum. The advantage of TEM is the loss of all water as it is equipped with a vacuum. The result in the photographing mode was analyzed. [14]

g) FTIR-spectral analysis for the drug loaded Pluronicmicelles:

The FTIR spectrum of the micelles was taken, by the pellet method. In this method, the micelle formulation on the basis of the best two formulations from the entrapment and loading capacity was selected and approximately 5 mg was mixed or say triturated with the 100-150 mg of KBr. A proper physical mixture was made through trituration but with a mild force as excessive force may cause the micelles to disrupt up. Then this mixture was placed in the cavity for disc preparation and was then subjected to compression through the compression machine. After that, by using the revert compression the disc was separated from the disc forming stand and was placed in the disc holder for FTIR examination. The disc along with the holder was placed in the FTIR instrument and was analyzed within 5000 cm-1 to 500 cm-1, in the same manner, the FTIR spectra of the polymer and drug physical mixture and one more formulation was taken and were analyzed for the variation. [21]

h) Thermal analysis of drug loaded nano-micelles:

The thermal analysis was carried on with the aid of Differential Scanning Calorimetry. In this method, the best acted on formulation from the FTIR was taken and 2mg near about of it was weighed, simultaneously 2 mg of drug and 2 mg of poloxamer were weighed and transferred one by one to the two holders of the DSC apparatus, and the procedure was started by setting the temperature range from -40 to 300 °C. The temperature was raised at a rate of 10 °C per minute, with aluminium serving as a reference. The curve was obtained and analyzed for the investigation of the poly-dispersity of the drug in the polymer. [19, 20]

i) X-Ray Diffraction of Nano micelle:

The FTIR and TEM formulations were sent for XRD analysis. A minimum of 1 gram of material was taken, and a slide of the nano micelle sample was created. The sample was then analyzed using XRD over a range of brag angles ranging from 0° to 180°. This range was selected to provide all of the possible peaks of the drug's diffraction spectra when x rays are bombarded on it. Similarly, the drug's XRD was performed by taking 1 gm of it, producing a slide, and then analyzing the drug from 0° to 180°. The differences in the peaks were investigated. If the final formulation is crystalline, the peaks will be sharper; otherwise, they will be curved and smooth. Smooth peaks are required for nano micelle because they ensure that the drug is completely dispersed in the polymer. [38, 39, 40]

j) HET-CAM Test:

The HET-CAM test is a modification of the Draize eye test that was performed in order to check whether the drug was not causing any inflammation or injury to the eye and is isotonic with the eye fluid. This test gives us a proper indication of the inflammation, injury, protein coagulation, and damage that a formulation will cause to the eye, as these membranes in the egg or the chorioallantois membrane are equivalent insensitivity to the layers of the eye.[28, 29]

k) Microbial testing:

The final formulation that was selected was sent for microbial testing. The testing was done for checking whether the formulation was able to inhibit the growth of the microorganism and cause their death or not. This was decided by preparing an agar medium plate by inoculating the microorganism S.aureus, and then checking for inhibition by nano micelle solution.[23, 24]

l) Stability studies

The reason behind stability testing is to see how an active substance or a formulation varies with the time and in accordance with the various other factors as a temperature and humidity and light. It also provides an idea of how the product-related factors show an impact on the chemical and physical properties of the API and the various additives or excipients involved in making a fine formulation, and in addition to it, we also come to know the effect of the packing material on the dosage form. This evaluation helps us to know whether the excipients prove harmful for the formulation by forming a reactive or a cause for degradation of the product.

The final formulation selected was incubated for a period of one month at the temp. and humidity for accelerated study (40 0C ± 2 °C / 75 % ± 5 % RH), after one month the formulation obtained was analyzed for the drug content and content uniformity through the UV-Vis spectrophotometry and FTIR.[25, 26, 41]

3. Results and Discussion:

A) Preformulation:

a) Solubility: The solutions were observed after 24 hrs, and the results are as in Table 2.

b) Melting point:

The melting point from the apparatus, was great venture to watch, the point of the correct melting was clearly visible and was identifiable, which is a limitation of other apparatus. The apparatus for the observation was set up in the range of 220-270 °C, and the result was found to be 244 °C. We can also say that melting was observed in a range of 242-245 °C.

c) FTIR:

The analysis through the Shimadzu FTIR was done, and the curve sharpening through various voltage or the modifications was done and the peaks were obtained. The peaks were marked with frequencies, and the frequencies obtained where tallied with standard and was checked for the purity of the compound as shown in Fig 2 & Table 3.

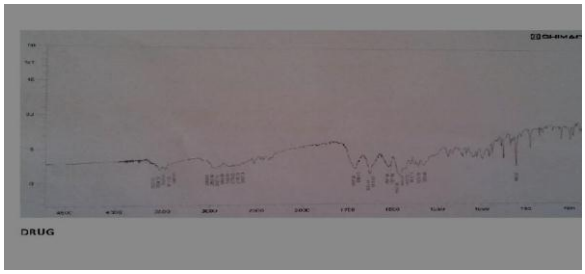

Fig 2: FTIR Spectra of Drug

d) UV-spectrophotometric analysis of drug:

The absorbance was found to be 289 nm as shown in the curve below. At this absorbance the curve showed a maximum absorbance through a peak and this absorbance can be considered a favourable absorbance for considering the further analysis through dilutions in STF and also this lambda max is verified through the literature published in regard of this drug and also through the various compendia's and formularies. Table 4 and Fig 3.

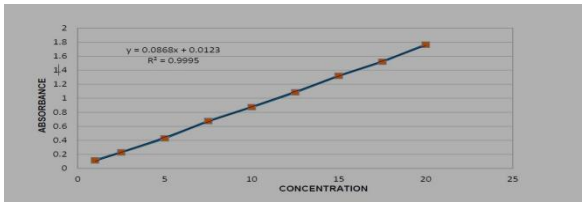

Fig 3: Calibration Plot of Drug in STF

e) Partition coefficient method:

The separated aqueous layer was subjected to UV analysis, and the absorbance was recorded. The absorbance was then kept in the equation to calculate the concentration and so the amount, this amount obtained through aqueous layer examination was subtracted through the total amount dissolved in the flask, the amount we got was the amount that went into the organic layer. Thus, the partition coefficient was calculated in the table 5 and calibration plot of drug in water in Fig 4.

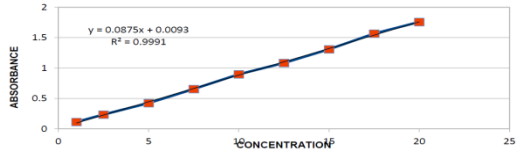

Fig 4: Calibration Plot of Drug in Water

C) Formulation evaluation result:

a) Drug entrapment:

The absorbance of the Nano micelle solution of 10 mg was measured in triplicate and then put into the equation together with all of the multiplication factors to obtain the exact concentration that was released from the nano micelle, and then kept in the formula to get the entrapment as shown in table 6.

The entrapment was considerably high, reaching 99%, indicating that the drug was highly and easily incorporated in the formulation, and the chitosan utilised did not interfere with the formulation release. The modification in entrapment was mostly influenced by the varying concentrations of PVA and Polox 407 used; even the amount loaded created a significant variation in entrapment. As a result, four formulations were chosen for further investigation.

b) Drug loading capacity

The absorbance for the Nano micelle solution of 10 mg after 24 hrs of magnetic stirring was noted in triplicate and drug content from the equation was kept in the formula to calculate the Loading Capacity as in Table 7.

The loading capacity was found as per the drug loaded and highly varied along the PVA and Polox 407 changes. The capacity was higher till 0.07% and even lower till 0.03%, so the ratio needed to be adjusted to get a formulation of high loading capacity.

So, on this basis even finally four formulations were selected for In-Vitro release study i.e., NM-4, NM-5, NM-7, NM-8.

c) In-vitro drug release:

The absorbance recording corresponding to each sample withdrawn at the predefined intervals was recorded using a UV-Vis Spectrophotometer to analyse the Nano micelle solution of 10 mg NM in STF. These absorbance values were obtained after sufficient dilution to obtain a result within the Beer – Lambert range. The drug's release was calculated and the pattern of release was studied, and it was observed that it showed a slow initial release till the 60 min, followed by a burst release and then a sustained release that reached its peak in the 7th hr. and cumulatively 100% in the 23rdor 24thhr., as shown in table 8.

Release of NM-8 shown in: Fig 5, 6, 7, 8, 9 and table 8.

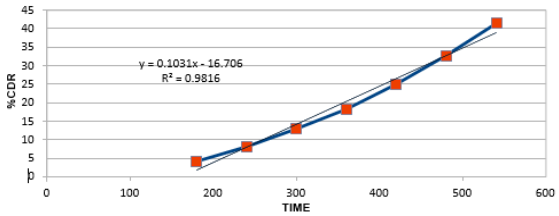

Fig 5: Zero Order Model for NM-8

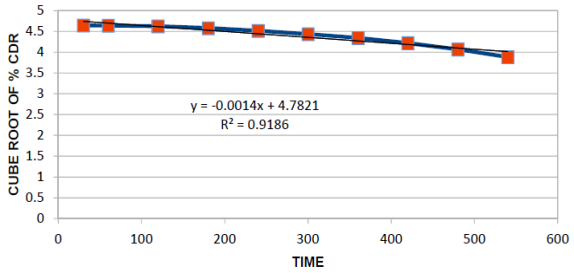

Fig 6: First Order Model for NM-8

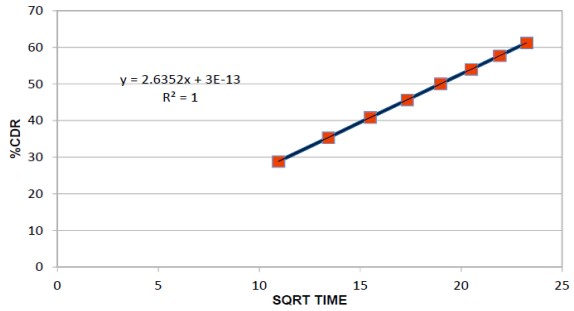

Fig 7: Higuchi Model for NM-8

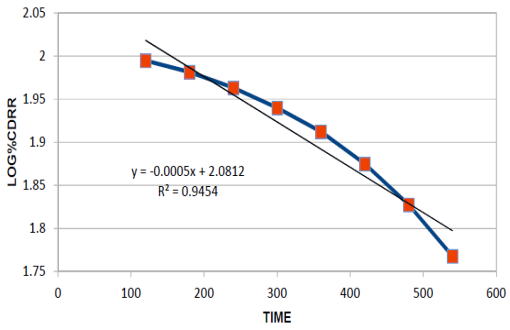

Fig 8: Hixson Crowell Model for NM-8

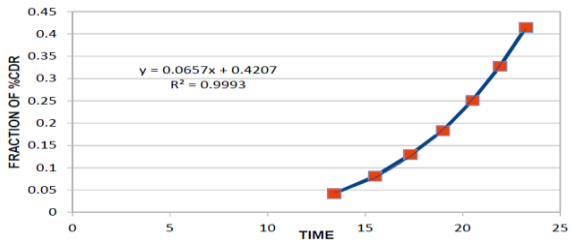

Fig 9: KorsemeyerPeppas Model for NM-8

Thus, the nano micelles composed of Pol. 407 as the main polymer showed a release that was based on first the erosion of the chitosan layer that provided it a positive charge, followed by the swelling of the hydrophilic tri-block copolymer and thus diffusion from the pores in the polymer base i.e., a 60 to 70 percent release in the first few hours and then a gradual release from the triblock till 8 hours and then a final high release in the 23rd hr. So, we can say that on the application of the nano micelle solution to the eye, due to chitosan it will bind up through the electrostatic interaction and then will give an initial release that will relieve the patient initially and will provide him a constant release after that till 8 to 9 hrs. So, the formulation will lower the usual frequency and the release will also be favourable for the patient.

d) Particle size, Zeta Potentialand Poly-dispersity Index

The particle size, zeta potential, and PDI were studied only for four formulations namely NM-4, NM-5, NM-7, and NM-8. Because the chitosan polymer produces a positive charge layer over it, the size acquired through this measurement was found to be somewhat greater than that reported only with the Poloxamer. This created layer is extremely helpful since it first erodes to release the drug, followed by subsequent release; however, this increases particle size. This layering is important because it helps in binding with the inner core of the eye, enhances nano micelle residence duration in the eye, and allows for delayed and prolonged action with no drug scarcity during treatment. Ifthis layer does not form, the particles may release the drug for a prolonged period of time than usual, and there may be a scarcity scene in the eye for the treatment of infection. As a result, this layering is essential. Now coming to the PDI, according to the literature for the ocular delivery the maximum PDI should be less than 0.25, as if more is seen then they will not act as a good carrier for ocular delivery and the size incensement will make the particle unsuitable for ocular delivery. Lastly, the Zeta Potential was found out through DLS and it was found out to be positive, thus assuring us that the particles are coated with chitosan and the particles will help in delaying the effect by binding in the core of the eye for a long duration and releasing the drug for the assumed 6 to 8 hours. The particle size along with the PDI and Zeta Potential was found to be as shown in table 9.

Thus, from the above values we can say that the formulation NM-8 produced more satisfactory result with particle size, potential and PDI as compared to other formulations.

e) Turbidity analysis:

In the turbidity analysis the solutions of nano micelle were made and were analysed. The transmittance was noted and turbidity was as shown in table 10.

We are administering the solutions for the eye, the eye being the most delicate organ needed for all sorts of verification. The solution intended should not be turbid because if it happens to be even translucence then it may cause the patient an impairment of vision. The nano solutions on storage for a longer duration cause aggregation, that's why these solutions need to be made just before administering, but if in solution form then even till 12 hrs nano micelle formulation showed great transparency in the solution keeping the turbidity below the level of 0.2, that is the best suited for the formulation. So, from this, we can say that the formulation NM-4, NM-5, NM-7, and NM-8, showed a better consistency and out of them NM-5 and NM-8 will not cause any blurriness case for the patient due to their lower value of turbidity till 12 hrs also.

f) TEM of the nano micelle formulation:

The formulation was subjected to TEM investigation, the results of which are depicted in the figure below. The particle size for NM-5 was 101 to 119 nm and 98.6 to 99.8 nm for NM-8, as indicated in the figure below. The measurements were taken at 40,000 magnifications and over a 500nm range. The nano micelle is depicted by the cloudy, less dense area, while the drug is depicted by the dark marked area. The drug was entrapped in almost every nano micelle, and the nano micelle obtained is almost oval or spherical in shape. The nano micelles generated as a result are clear; the point now is the surface modification. The surface-modified nano micelle must be larger in size, with dimensions ranging from 100 to 125nm. This is due to the chitosan layer's modification of the surface, which helped in providing a positive charge to the sphere. This charge will be produced when the solution is formed and will allow the drug to bind in the eye or in the layers through electrostatic interaction, as the eye atmosphere or layers carry a negative charge; this charge is the main basis for the nano micelle through chitosan formulation. So, we can say that the nano micelles from the formulation NM-8, are properly

chitosan-coated which can be even seen in Figures 10 & 11, so NM-8 can opt for DSC, XRD, HET CAM and Microbial testing.

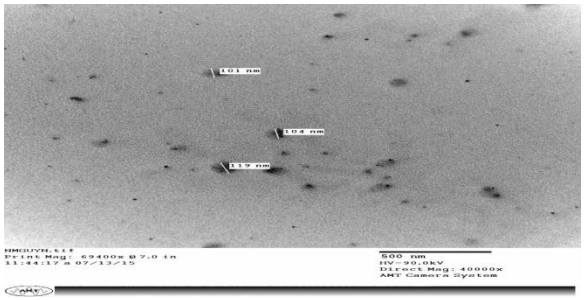

Fig 10: TEM of NM-5

Fig 11: TEM of NM-8

g) FTIR-spectral analysis for the drug loaded Pluronic micelles:

The FTIR spectra of NM-5 and NM-8 were obtained through this method, were analysed, and the peaks that declined or increased were checked or compared through the FTIR spectra of drug.

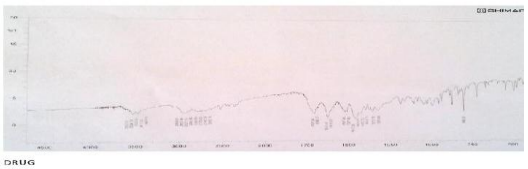

Fig 12: FTIR spectra of drug

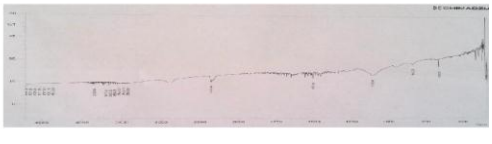

Fig 13: FTIR OF NM-5

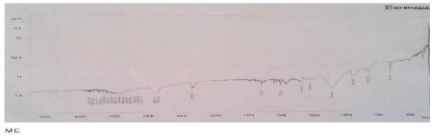

Fig 14: FTIR OF NM-8

The number of peaks increased that showed us that the drug was completely in the formulation, no more peaks for alcohol or less prominence in the -OH peak showed us that the alcohol that was used for the formulation as the dispersing agent has been evaporated, and is not present in the formulation. The peaks are as mentioned in the table 11.

The peaks showed in fig 12 were almost equivalent to that of the drug, proving that the drug is nicely dispersed in the polymer. The formulation NM-8 fig 14 showed better peaks than the formulation NM-5 fig 13, so for DSC, XRD, HET CAM and Microbial testing it was selected.

h) Thermal analysis of drug loaded nano-micelles:

Thermal analysis was performed, and the results are given in figures 15, 16, and 17. The melting point of the poloxamer was determined to be 47.10 °C, while that of the drug was determined to be 243.97 °C. The melting points of the peaks were determined and verified to be correct. Moisture was detected in the drug DSC curve by a peak at 103 °C. The DSC of the nano micelle revealed a single peak at 48.11 °C and no peak at 47.10 °C or 243°C, indicating that the drug is totally entrapped in the polymer and the drug peak has shifted a long way. The large shift is due to the fact that the drug is present in extremely small amounts in comparison to the polymer; even the literature showed that in the case of Poloxamer. high concentration, the shift is always quite less, and here the percentage is 0.01, indicating a very small shift. The curve shows only a small bulge near 240 °C, not a peak. This indicates that the drug is well dispersed in the polymer and that the formulation was successful. As a result, we may conclude that the drug is easily carried by the polymer, and additional investigation can be conducted using the polymer and drug XRD spectra to determine the drug's dispersion in the polymer matrix.

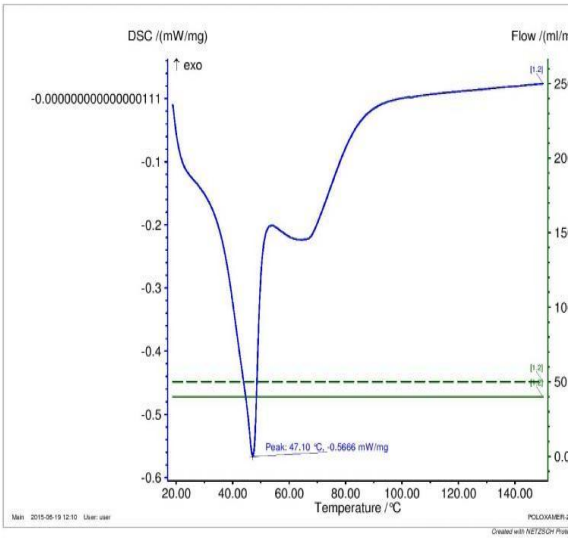

Fig 15: DSC OF POLOXAMER.

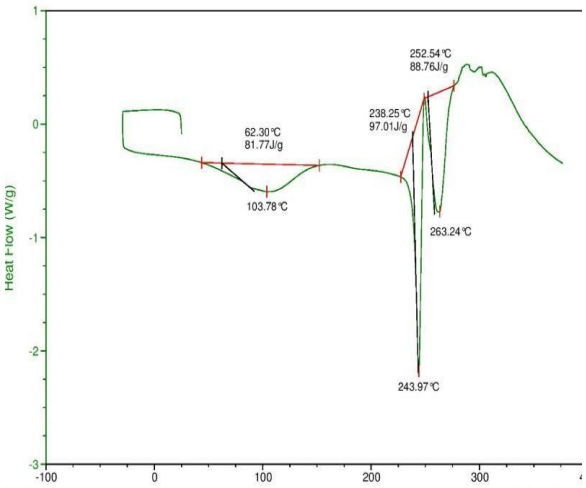

Fig 16: DSC of drug

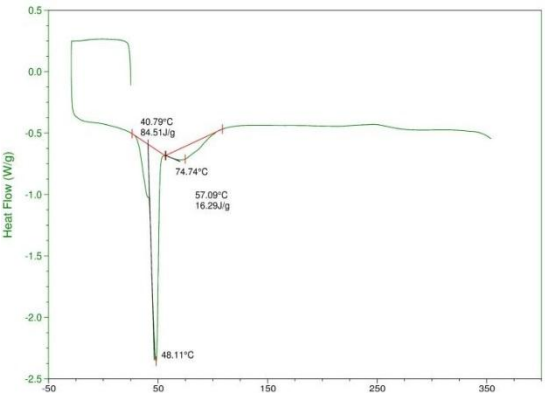

Fig 17: DSC OF nano micelle

i) X-Ray Diffraction of nano micelle:

The resulting nano micelle was examined using a powder X-Ray diffractometer. The drug powder diffraction was compared to that of the dried nano micelle. The peaks in the drug spectra (fig 18) were quite pointed and sharp, indicating that the drug is the only one contained in it. However, when the combination was examined in the X-RD apparatus, the sharp peaks changed into curved peaks (fig 19). This indicated that the drug was properly incorporated into the polymer.

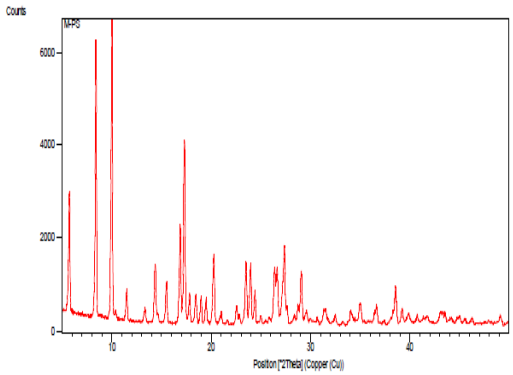

Fig18: XRD OF Drug

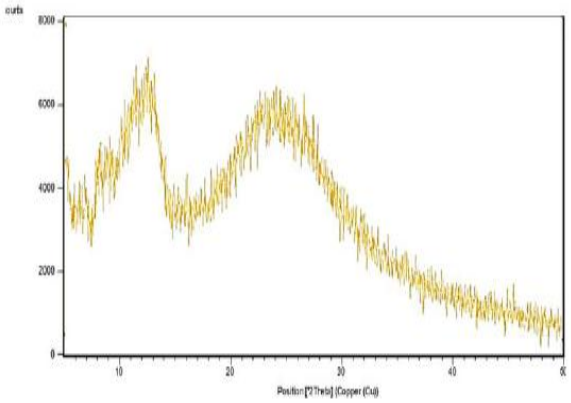

Fig 19: XRD of nano micelle

j) HET-CAM test:

The tests for chorioallantois membrane were performed on three eggs and the results were satisfactory for the formulation when compared with the negative and positive control through the saline and surfactant. The saline does not show any inflammation or any other effect in the duration of 14 hours. The drug showed a little but as compared to the positive control by surfactant the inflammation or we can say that the redness produced was less as shown in fig 20(a), 20(b), 20(c), 20(d), 20(e) and 20(f).

Fig 20(a): Initial Image of Membrane for Saline

Fig 20(b): Final Image of Membrane for Saline

Fig 20(c): Initial Image of Membrane for Drug

Fig 20(d): Final Image of Membrane for Drug

Fig 20(e): Initial Image of Membrane for Surf.

Fig 20(f): Final Image of Membrane for Surf.

Thus, it was found and proved that the formulation did not cause any inflammation, protein degradation; it is isotonic and can be used for the human eye.

k) Microbial Testing:

The microbial test was performed and it was found that the drug was able to kill and inhibit the colony formation, and it was releasing the drug in a good amount that was appropriate for the usage as an antibiotic. The colony inhibition increased with the amount or concentration, and thus the various concentrations that was employed was equivalent to that of the release of the formulation for C-max and thus the inhibition will be there at the site. Hence from the lower image starting from the center in low concentration and going in a clockwise direction, we can say that the drug has a great spectrum of action for the microorganism S. aureus that was one of the major causative agents for many eye infections and inflammation.

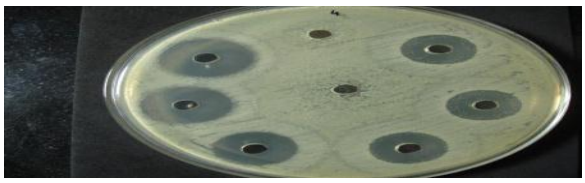

Fig 26: Microbial Testing slide of Drug

D) Stability studies:

The stability studies were performed and the result are decided on the basis of the drug content and FTIR spectra.

a) UV-VIS analysis:

For UV determination, 100 mg of nano micelle were weighed from the batch kept for stability studies and a solution of them were made in STF on magnetic stirring. The solutions were analysed and the absorbance was noted and the results are in table 12.
The amount obtained was near to that of theoretical expected with that of a standard deviation of 0.003 that is within the limit, and the %RSD below 0.2%.

b) FTIR analysis:

The FTIR spectra of the batch that was kept was analysed for the FTIR and the results are as shown in the table 13.

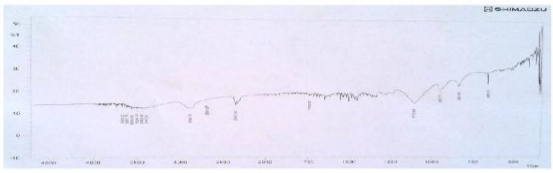

Fig 27: FTIR-Analysis of the Stability Batch

Thus, we can say that the formulation did not degraded and it is still quiet near to that of the standard formulation prepared one month before.

Analysis by QBD

Factorial Design for three factors at two levels with -1 and +1 equivalent to a 2^3 factorial design was chosen as the experimental design. To investigate the factors systematically, a full factorial design was employed. The effect on loading capacity (Loading) was observed to be significant by ANOVA and the polynomial equation was found as follows:
Loading = 0.07562 – 0.00375 Poloxamer – 0.000125 Drug – 0.007125 PVA + 0.000875 Poloxamer * Drug –0.001625 Poloxamer *PVA +0.00625 Drug*PVA - 0.002875 Poloxamer* Drug* PVA.

3d plots show us the better effect of each one's concentration on the loading capacity of the drug.
The effect on % Drug Entrapment (ENTRAPMENT) was observed to be significant by ANOVA and the polynomial equation was found as follows:
Entrapment= 87.33 – 2.395 Poloxamer + 0.7605 Drug – 5.539 PVA + 0.4165 Poloxamer *Drug – 0.9550 Poloxamer * PVA + 0.06050 Drug * PVA – 3.995 Drug * Poloxamer * PVA.

The 3D curves are:

A) **First curve**

Curve 1 Conclusion:
From this curve we conclude that as poloxamer increases the entrapment increases. The increase in drug amount also shows a positive effect. (Fig 28)

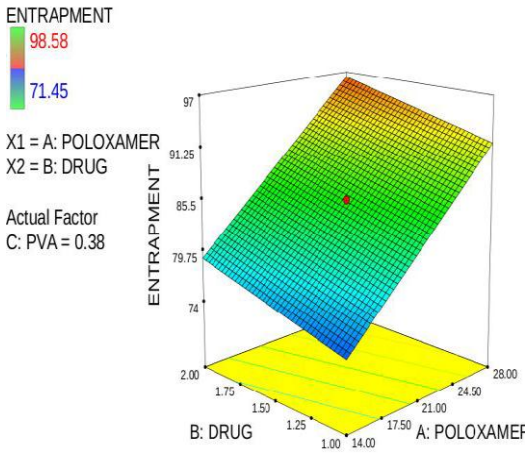

Fig 28: Effect of poloxamer and drug on entrapment

B) **Second curve**

Curve 2 Conclusion:
From this curve we conclude that as poloxamer increases the entrapment increases, PVA on the other hand also pose a great impact, as PVA increases the entrapment increases. (Fig 29)

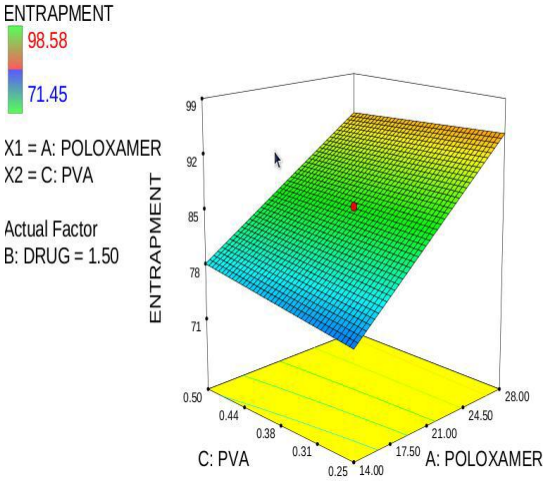

Fig 29: Effect of poloxamer and PVA on drug entrapment

C) **Third curve**

Curve Conclusion: On the basis of this curve, it was found that the loading was directly affected by the poloxamer and drug concentration. As the poloxamer concentration increases it enables more loading capacity of the polymer, but as it decreases it lowers the loading capacity and also the drug amount directly influence it. (Fig 30)

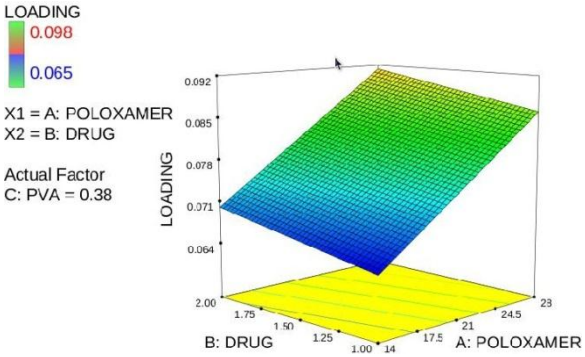

Fig 30: Effect of Poloxamer and Drug on Loading

D) **Fourth curve**

Curve Conclusion: The poloxamer and the PVA are directly having influence on the loading capacity. The drug being constant here we can say that Poloxamer is having a great impact on the loading capacity and its amount directly influence it but the PVA amount causes the loading to increase but not as much as the poloxamer, but still the formulation with increasing PVA provides the polymer with more loading capacity. (Fig 31)

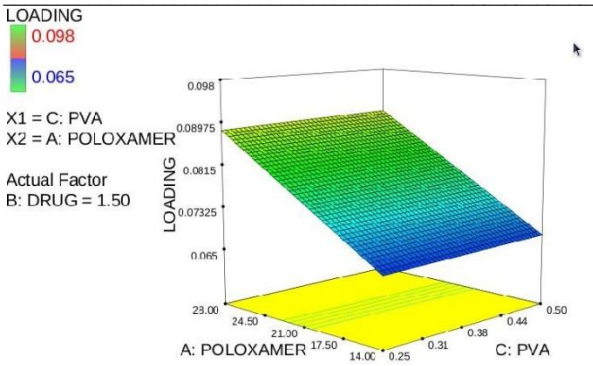

Fig 31: Effect of Poloxamer and PVA on Loading

Conclusion:

Thus, through this work we were able to create a formulation that showed:

i. Efficient drug loading and encapsulation was far-far efficient than other drugs chosen out.
ii. The drug in formulation formed clear solution, and even on storage at the desired temperature the formulation did not denatured.
iii. The osmolality and pH were obtained as desired.
iv. The size of the formulation was obtained in the limits of 90-110 nm which was quite efficient for the formulation.
v. The drug was properly poly-dispersed in the formulation which was proved through the XRD, FTIR and TEM analysis of the formulation.
vi. The negative charge developed gives us an assurance that the electrostatic binding can be obtained which may increase up the drugs residence time.
vii. The applied QBD gave us an idea initially only that out of all NM-8 and NM-5 were efficient, but through the analysis here we have an exact idea that the formulation NM-8 is most efficient and the regression which was of 0.995 from 0.992 of NM-5 is most proper conveyed message through the formulation.

Conflicts of interest

There are no conflicts to declare.

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Table 1: Formulation according to 2^3 Factorial Design

S.No.	Formulation name	Polymer Concentration	Drug Concentration	PVA concentration
1	NM-1	14.00%	2 mg	0.25%
2	NM-2	14.00%	2 mg	0.50%
3	NM-3	28.00%	2 mg	0.50%
4	NM-4	28.00%	1 mg	0.50%
5	NM-5	28.00%	2 mg	0.50%
6	NM-6	14.00%	1 mg	0.50%
7	NM-7	28.00%	2 mg	0.25%
8	NM-8	28.00%	1 mg	0.25%

Table 2: Solubility Analysis

S.No.	Solvent	Drug amount	Solubility
1	Water	10mg	Soluble
2	Ethanol	10 mg	Freely soluble
3	Acetone	10mg	Slightly Soluble
4	STF	10mg	Practically Soluble
5	Propanol	10 mg	Sparingly Soluble
6	Methanol	10 mg	Soluble

Table 3: FTIR Interpretation of Drug

S.No.	Functional group	Reported frequencies (cm^{-1})	Observed frequencies (cm^{-1})
1	F	1400-1000	1351
2	C=O in ketones	1720-1700	1707
3	C=O in carboxylic acids	1710-1690	1696
4	N-H	3500-3310	3472
5	-OH, in carboxylic acids	1440-1400	1423, 1430
6	$-OCH_3$	2850-2600	2770
7	NH_3^+in hydrochlorides	1530-1490	1507, 1517
8	C= C	1610-1620	1616
9	CH_2 in aliphatic compounds	1475-1450	1457
10	CH_2 out of plane deformation	790-810	803
11	C-N in primary amides	1420-1400	1418
12	CH_2 Stretching Vibration	3000-2800	2966
13	CH= CH	1680-1600	1624
14	CH_3 in aliphatic compounds	1465-1440	1457

Table 4: Drug Calibration Plot

S.No.	Concentration	Absorbance
1	1	0.115
2	2.5	0.228
3	5	0.429
4	7.5	0.675
5	10	0.874
6	12.5	1.085
7	15	1.32
8	17.5	1.521
9	20	1.765

Table 5: Partition Coefficient Table

S.No.	Absorbance of Aqueous layer	Conc. mcg/ml	Contentinaqueous layer (mg)	Content in organic layer (mg)	Partition coefficient	LOG P	Standard deviation
1	0.239	2.62484	6.56211	3.43789	0.52390	-0.28075	
2	0.238	2.61342	6.53354	3.46646	0.53056	-0.27526	0.0038470682
3	0.239	2.62484	6.56211	3.43789	0.52390	-0.28075	

Table 6: Drug Entrapment

S.NO.	Form. No.	Absorbance	Drug content	Weight of drug taken	Drug entrapment	Average	Standard deviation
		0.026	0.0063		80.9827	84.9120	
1	NM-1	0.027	0.0068	0.0078	86.8767		3.4029
		0.027	0.0068		86.8767		
	NM-2	0.027	0.0068		86.6770	82.7568	
2		0.026	0.0063	0.0078	80.7967		3.3950
		0.026	0.0063		80.7967		
	NM-3	0.028	0.0073		85.1950	86.9993	
3		0.028	0.0073	0.0085	85.1950		3.1250
		0.029	0.0077		90.6077		
	NM-4	0.021	0.0040		96.6225	92.9374	
4		0.021	0.0040	0.0042	96.6225		6.3827
		0.02	0.0036		85.5673		
	NM-5	0.02	0.0036		91.2148	99.0714	
5		0.021	0.0040	0.0039	102.9997		6.8040
		0.021	0.0040		102.9997		
	NM-6	0.019	0.0031		72.1906	79.3311	
6		0.021	0.0040	0.0043	93.6121		12.3677
		0.019	0.0031		72.1906		
	NM-7	0.028	0.0073		92.9967	89.0578	
7		0.027	0.0068	0.0078	87.0884		3.4112
		0.027	0.0068		87.0884		
	NM-8	0.02	0.0036		86.3966	90.1174	
8		0.021	0.0040	0.0041	97.5590		6.4446
		0.02	0.0036		86.3966		

Table 7: Drug loading Capacity

S.NO.	Form No.	Absorbance	Drug content	Weight of drug taken	Drug entrapment	Average	Standard deviation
		0.026	0.0063		0.0633		
1	NM-1	0.027	0.0068	10.0000	0.0679	0.0664	0.0027
		0.027	0.0068		0.0679		
		0.027	0.0068		0.0679		
2	NM-2	0.026	0.0063	10.0000	0.0633	0.0648	0.0027
		0.026	0.0063		0.0633		
		0.028	0.0073		0.0725		
3	NM-3	0.028	0.0073	10.0000	0.0725	0.0740	0.0027
		0.029	0.0077		0.0771		
		0.021	0.0040		0.0403		
4	NM-4	0.021	0.0040	10.0000	0.0403	0.0387	0.0027
		0.02	0.0036		0.0357		
		0.02	0.0036		0.0357		
5	NM-5	0.021	0.0040	10.0000	0.0403	0.0387	0.0027
		0.021	0.0040		0.0403		
		0.019	0.0031		0.0310		
6	NM-6	0.021	0.0040	10.0000	0.0403	0.0341	0.0053
		0.019	0.0031		0.0310		
		0.028	0.0073		0.0725		
7	NM-7	0.027	0.0068	10.0000	0.0679	0.0694	0.0027
		0.027	0.0068		0.0679		
		0.02	0.0036		0.0357		
8	NM-8	0.021	0.0040	10.0000	0.0403	0.0372	0.0027
		0.02	0.0036		0.0357		

Table 8: Release of Nano micelle NM-8

S.No.	Time	Avg. abs	Drug content	Drug release	%DR	CDR	%CDR	%CDRR	LOG(%CDRR)
1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	100.000	2.0000
2	30.0000	0.0070	0.0021	0.0001	0.7478	0.0001	0.0957	99.9043	1.9996
3	60.0000	0.0070	0.0021	0.0001	0.7478	0.0001	0.1914	99.8086	1.9992
4	120.0000	0.0090	0.0229	0.0007	8.1882	0.0008	1.2394	98.7606	1.9946
5	180.0000	0.0130	0.0646	0.0019	23.0692	0.0028	4.1921	95.8079	1.9814
6	240.0000	0.0150	0.0854	0.0026	30.5097	0.0053	8.0970	91.9030	1.9633
7	300.0000	0.0170	0.1063	0.0032	37.9501	0.0085	12.9543	87.0457	1.9397
8	360.0000	0.0180	0.1167	0.0035	41.6704	0.0120	18.2877	81.7123	1.9123
9	420.0000	0.0210	0.1479	0.0044	52.8311	0.0164	25.0496	74.9504	1.8748
10	480.0000	0.0230	0.1688	0.0051	60.2716	0.0215	32.7639	67.2361	1.8276
11	540.0000	0.0250	0.1896	0.0057	67.7121	0.0272	41.4304	58.5696	1.7677
12	1200.000	0.0310	0.2521	0.0076	90.0335	0.0348	52.9538	47.0462	1.6725
13	1260.000	0.0310	0.2521	0.0076	90.0335	0.0423	64.4773	35.5227	1.5505
14	1320.000	0.0310	0.2521	0.0076	90.0335	0.0499	76.0008	23.9992	1.3802
15	1380.000	0.0320	0.2625	0.0079	93.7537	0.0578	88.0004	11.9996	1.0792

Table 9: Particle size, Zeta Potential and Poly Dispersity Index

S.No.	Formulation Name	Particle Size	Zeta Potential	Poly-Dispersity Index
1	NM-4	101nm + 6	8.9 + 0.5	0.117 + 0.006
2	NM-5	95 nm + 3	7.8 + 0.1	0.156 + 0.002
3	NM-7	98 nm + 7	11.5 + 0.3	0.125 + 0.003
4	NM-8	94 nm + 2	8.1 + 0.2	0.165 + 0.002

Table 10: Turbidity Analysis

S.NO.	Avg.% trans. (Zero HR)	Turbidity	Avg.% trans (6 HR)	Turbidity	Avg.%trans. (12 HR)	Turbidity
NM-4	96.7	0.033	93.5	0.065	91.2	0.088
NM-5	98.2	0.018	95.4	0.046	90.4	0.096
NM-7	96.1	0.039	91.2	0.088	89.8	0.102
NM-8	97.4	0.026	92.5	0.075	90.5	0.095

Table 11: FTIR interpretation of product for product

S.NO.	Functional Group	Reported Frequencies (cm^{-1})	Formulation depicting	Observed Frequencies (cm^{-1})
1	2990-2850	-$CH_3$and-CH_2in aliphatic comp	POL	2890
2	2350-2750	-NH_3^+in hydro halides	DRUG AND POL	2360
3	1760-1740	C=O in alpha keto esters	DRUG AND POL	1739
4	1565-1475	COO^- in carboxylic acid salts	DUG	1558
5	1515-1485	BENZENE RING	DRUG	1507
6	1465-1440	CH_2in aliphatic compounds	DRUG AND POL	1457
7	1475-1450	CH_3 in aliphatic compounds	DRUG AND POL	1457
8	1400-1000	F	DRUG	1349
9	1200-1015	C-OH in alcohols	POLYMER	1114
10	1000-950	$CH=CH_2$	DRU AND POL	964
11	790-850	$CH=CR_2$	POLYMER	841

Table 12: UV-Analysis for Stability Study

S.No.	Formulation	Absorbance	Drug Amount	Drug Amount	S.D.	%RSD
			THEORITICAL	PRACT ICAL		
1		0.058	0.06mg	0.055	0.0035118846	0.002
2	NM-8	0.059	0.06mg	0.058		
3		0.057	0.06mg	0.051		

The amount obtained was near to that of theoretical expected with that of a standard deviation of 0.003 that is within the limit, and the %RSD below 0.2%.

Table 13: FTIR-Analysis for Stability Study

S.No.	Functional Group	Reported Frequencies (cm^{-1})	Formulation Depicting	Normal Form. Frequencies (cm^{-1})	ACCE. Form. Frequencies (cm^{-1})
1	$-CH_3$ and $-CH_2$ in aliphatic comp.	2990-2850	Polymer	2890	2815
2	$-NH_3^+$ in hydro halides	2350-2750	Drug & Polymer	2360	2315
3	C=O in alpha keto esters	1760-1740	Drug & Polymer	1739	1725
4	COO^-in carboxylic acid salts	1565-1475	Drug	1558	1442
5	Benzene ring	1515-1485	Drug	1507	1501
6	CH_2 in aliphatic compounds	1465-1440	Drug & Polymer	1457	1456
7	CH_3 in aliphatic compounds	1475-1450	Drug & Polymer	1457	1456
8	F	1400-1000	Drug	1349	1342
9	C-OH in alcohols	1200-1015	Polymer	1114	1110
10	$CH=CH_2$	1000-950	Drug & Polymer	964	961
11	$CH=CR_2$	790-850	Polymer	841	840